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ORIGINAL PAPER

Natural range expansion and human-assisted introduction leave different genetic signatures in a hermaphroditic freshwater snail

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Abstract Colonization events like range expansion or biological invasions can be associated with population bottlenecks. Small population size may lead to loss of genetic diversity due to random genetic drift, to loss of heterozygosity due to increased inbreeding and should leave a signature on the genetic polymorphism and genetic structure of populations. The mating system might additionally influence the outcome of such a process. Here, we compare invasive and native populations of the hermaphroditic freshwater snail *Lymnaea stagnalis*. In the native range we included populations that were ice-free during the last glaciation period and populations that were glaciated and are located at the edge of the species' native distribution range. The microsatellite data show substantial loss of genetic variation in the introduced range and no signs of high propagule pressure or admixture. The expressed polymorphism was so low that mating system analysis was not possible. In the native region, all populations display strong levels of differentiation (global F_{ST}: 0.341) independent of colonization history and exhibit no significant pattern of inbreeding. However, the populations in more recently colonized habitats show diminished genetic diversity. Overall, these results illustrate how dramatic the reduction in genetic diversity can be for hermaphroditic animals and that gene flow in the native range can be surprisingly low despite short distances.

Keywords Colonization · Range expansion · Invasion · Genetic drift · *Lymnaea stagnalis* · Gastropod

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Introduction

Various traits such as dispersal ability, mating system, tolerance to environmental stress and adaptability to new environmental conditions are important for successful colonization. Many of these traits are expected to have a genetic basis and the amount of genetic variation should be important for adaptation to the new environment. Genetic diversity is also the main parameter that is affected by population bottlenecks (Nei et al. 1975), which are often associated to colonization events like range expansion or biological invasions. Hence, in many colonization events the new gene pool for future generations might be founded by a few individuals only and the new population should be encumbered with reduced genetic diversity. Small population size also makes the emerging population vulnerable to biparental inbreeding and genetic drift, which is expected to erode the standing genetic variation even further.

Natural range expansion and invasion events can be seen as two extremes of a colonization process, but they differ in some substantial points. The loss of genetic diversity is expected to be less drastic for a newly founded population in a range expansion. It will be buffered by gene flow, because the population is part of a metapopulation, where distances to the source population are generally smaller than in an invasion event. The occurrence of non-native species is often due to human assisted introduction or spread over larger distances and across natural barriers. Both deliberate and unintentional introductions can vary in the number of individuals introduced and in the number of events. Together they define the propagule pressure which is an important variable to predict the genetic composition of an invasion. Another difference can be seen in the time scale: range expansion is a slow and progressive process, but biological invasions are often characterized by rapid and punctual actions.

The mating system can be one of the key factors for successful establishment in both scenarios. For dioecious and gonochoristic species the availability of mating partners is indispensable, which limits the lowest possible number of colonizing individuals to one female and one male, or to one sperm-carrying reproductive female that reproduces successfully and also produces male offspring. In alternative mating systems, like in self-compatible hermaphrodites and asexual organisms, a single individual can establish a new population and provide reproductive assurance. Unisexuality was observed early on in invertebrates to occur at the edge of a species distribution where the most recent range expansions have taken place (Vandel 1928). In plants of the same family, self-fertilizing species frequently occur on islands or at the boundaries of the species' distribution range, a pattern which is known as Baker's law (Baker 1955; Stebbins 1957). The latter two reproductive modes ensure offspring without a mating partner and are therefore often associated to successful colonization or invasion (Van Kleunen et al. 2008; Burns et al. 2011; Hörandl 2011). But establishment through a single individual is also the most extreme form of a population bottleneck, and will magnify the putative costs due to the loss of genetic diversity.

Here we examine the post-invasion reduction of genetic diversity and compare it to the signature of post-glacial range expansion in a hermaphroditic freshwater snail. Although the genetic effects of colonization events are well documented, not many studies exist for aquatic hermaphroditic species (Roman and Darling 2007). Due to their ability to self-fertilize these snails can establish a population by a single individual, and could therefore show an extreme genetic signature of a population bottleneck. Freshwater pulmonates represent an interesting group for studying this, especially as many species of this group are successful invaders in different parts of the world. This group pre-dates the Jurassic age

and expresses various mating systems (Dillon 2000). At the end of the 19th century, *L. stagnalis* was purposefully introduced to New Zealand by the Acclimatization Societies to support the diet of trout (Hutton 1881, 1884). The number of individuals or the number of actual introduction events is unknown, but the source population(s) most likely originated from England (Kopp, unpublished data). Meanwhile, the snail has established itself successfully, forming abundant populations in numerous lakes in New Zealand. *L. stagnalis* is considerably larger in size than any of the native freshwater snails, therefore resource competition with the native snail community cannot be excluded, although there is no evidence for *L. stagnalis* replacing any native gastropod species in New Zealand.

We examined the genetic diversity and genetic structure of *L. stagnalis* in the introduced and native range. Within the native range, we compared populations from regions that were ice-free (lowlands of Switzerland) or glaciated (Finland) during the last European ice age (ca. 30,000–10,000 years ago). Our comparison of native areas that differ in their post-glacial history allows comparison of (a) how much the level of genetic diversity among populations in the native range varies, and how this variation is structured with respect to the biogeographic history of the populations. The comparison of population genetic characteristics of these native areas with the invaded range should reveal (b) how much diversity is found in the introduced New Zealand populations today, and (c) if the mating system of the populations differs in the native range when compared to the introduced range.

Materials and methods

Species, sampling and genetic analysis

The freshwater snail *Lymnaea stagnalis*, is native to the holarctic region where it occurs in slowly flowing and stagnant water bodies with ample aquatic vegetation. As a simultaneous hermaphrodite, it can reproduce through self-fertilization or outcrossing. Earlier studies report that *L. stagnalis* individuals prefer to outcross if possible, and that they are able to store and use received sperm during approximately 3 months (Cain 1956; Coutellec-Vreto et al. 1994). On the other hand natural populations show relatively high inbreeding coefficients, possibly indicating a significant proportion of self-fertilization (Puurtinen et al. 2004).

Adult individuals of *L. stagnalis* (Gastropoda, Pulmonata, Basommatophora) were collected from 13 populations in the wild (Table 1). Populations sampled in the native range came from four temperate pond populations in Switzerland and four lake populations in Finland. Although we suspected England to be the source of the deliberate introduction to New Zealand, we did not manage to collect large enough samples for population genetic analyses despite our sampling efforts in the UK. These were encumbered by the fact that many ponds in England were formed relatively recently as a result of subsidence after the coalmining stopped in the 1920s. We therefore focus on the genetic signature of the two geographic extremes of the natural range expansion after the last glaciation. In the introduced range, we collected from four lake populations and a pond in Queenstown Botanical Gardens in New Zealand. Individuals were brought to the lab alive, where the head was dissected and briefly boiled in order to remove excessive mucus and then stored in 85% ethanol.

We amplified 15 microsatellite loci in total, nine of which were already available [GenBank Accession No. AY225955-AY225963 (Knott et al. 2003); please note that the

Table 1 Location of 13 *L. stagnalis* populations

Country	Population	Code	Coordinates	
Switzerland	Räubrichersee	RS	N 47° 36′	E 8° 40′
Switzerland	Brandholz	BH	N 47° 16′	E 8° 17′
Switzerland	Eschenberg	EB	N 47° 28′	E 8° 44′
Switzerland	Schlieren	SC	N 47° 23′	E 8° 27′
Finland	Jaurakkajarvi	JA	N 65° 09′	E 27° 39′
Finland	Viinimäki	VM	N 62° 34′	E 25° 41′
Finland	Korentojärvi	KO	N 65° 24′	E 27° 21′
Finland	Iso-Salminen	IS	N 64° 43′	E 27° 45′
New Zealand	Lake Alexandrina	AL	S 43° 58′	E 170° 26′
New Zealand	Lake Selfe	SE	S 43° 14′	E 171° 30′
New Zealand	Lake Heron	HE	S 43° 28′	E 171° 09′
New Zealand	Lake Hayes	HY	S 44° 58′	E 168° 48′
New Zealand	Queenstown	QT	S 45° 02′	E 168° 39′

Populations from Switzerland and Finland belong to the native distribution range, whereas populations in New Zealand are non-native

publication of Knott et al. (2003) in Table 1 displays the direction of reverse primers by mistake as 3′–5′)]. We developed six additional loci [EF208747-EF208752] by using four enriched libraries. First, we constructed two libraries enriched with (CA) and (GA) repeats following the protocol by Tenzer et al. (1999) and Gautschi et al. (2000). In the second approach the genomic libraries were enriched twice following the protocol of Hale et al. (2001). The insert for 95 clones in the range of 300–700 bp was sequenced and we were able to design 50 primer pairs for regions that showed more than 8 repeat motifs with the program macvector 7 (Accelrys) and Primer 3 (Rozen and Skaletsky 1998). In both approaches we used the BigDye Terminator Ready Reaction kit (Applied Biosystems) and analysed sequences on an ABI Prism® 3730 and 310 automated sequencer, respectively. Six out of the 50 primer pairs amplified and showed polymorphism.

Subsequent DNA extractions for the population study were done with BioRad Chelex® 100 (Walsh et al. 1991) from the collected tissue samples. Six microsatellite loci [GenBank Accession No. EF208747-EF208752] were amplified in 10 µl reactions with 1× Taq buffer [16 mM $(NH_4)_2SO_4$, 67 mM Tris–HCl, 0.01% Tween-20], 2.0 mM $MgCl_2$, 0.4 mM of each dNTP, 0.2 or 0.4 µM of each primer, 0.5 U *Taq* (Bioline) and 5–10 ng template DNA under the following conditions: 95°C for 12 min, 10 cycles of 94°C for 15 s, 53°C for 15 s, 72°C for 15 s, followed by 30 cycles of 89°C for 15 s, 51°C for 15 s, 72°C for 15 s, then 72°C for 30 min. The two loci 2k27 [EF208747] and 2k33 [EF208749] produced better results using only 0.2 µM of each primer. PCR products were analyzed on an ABI Prism® 310 Genetic Analyzer and alleles were identified using the GENESCAN® software (PE Applied Biosystems). More details on these loci can be found in Table 4 in the appendix.

The remaining 9 loci (Knott et al. 2003) were amplified in a total volume of 15 µl per reaction with 0.8× (or 1.2× for loci A2, B4 and A16) Reaction Buffer [1× = 15 mM Tris–HCl, 30 mM KCl, 5 mM $(NH_4)SO_4$, 2.5 mM $MgCl_2$, and 0.02% BSA], 200 µM dNTPs, 0.6 µM of each primer, 0.2 mg BSA, 0.4 U Dynazyme II Hotstart DNA polymerase (Finnzymes) and 5–10 ng of DNA with the following temperature cycling profile: a 10 min initial denaturing step at 94°C followed by 25 cycles of 20 s at 94°C for, 20 s at 55°C, 30 s at 72°C, another 10 cycles with 30 s at 89°C, 20 s at 54°C, 40 s at 72°C and a

final extension step at 72°C for 20 min. PCR products were analyzed on an ABI Prism® 3130xl Genetic Analyzer and alleles were identified using the GeneMapper software version 4.0 (Applied Biosystems).

Due to presence of null alleles we present an analysis based only on nine loci for which reliable analysis was possible. We encountered amplification failure at other loci where individuals did not amplify after several attempts. In the initial analysis we found highly variable inbreeding coefficients among loci although any level of self-fertilization should affect each locus in a similar way. Taken together with amplification failure we assumed that our microsatellite dataset contained null alleles. Null allele detection programs (e.g. Microchecker) assume random mating, which is easily violated in hermaphroditic organisms that may self-fertilize. To be conservative in our analysis, we therefore decided to exclude five loci from the analysis based on the rate of amplification failure and the highly variable inbreeding coefficients. The excluded loci showed amplification failure in the range of 9.4–14.6% and two to five loci had inbreeding coefficients higher than 0.5. We also ran our data through Microchecker to crosscheck our exclusion procedure. The program found four of the five loci to contain null alleles in at least four of the eight native populations. The excluded loci are: A16, A102, A112, B4, B117 (Knott et al. 2003) and 2k46 [EF208751]. Locus B19 (Knott et al. 2003) generated a strong non-specific amplification pattern and was also excluded from the dataset. We included the locus A2 (Knott et al. 2003), although it showed complete amplification failure in the population SC but was otherwise consistent. We are confident that our decision to exclude these loci is conservative as the results of the reduced data set tell the same story as the full data set but with lower numbers.

Statistical analysis

We tested the independence of loci by calculating genotypic disequilibrium and Hardy–Weinberg equilibrium with Genepop, version 3.4 (Raymond and Rousset 1995). We used Fisher's exact test and a subsequent Markov chain method to estimate unbiased *P*-values (Guo and Thompson 1992). Number of polymorphic loci, mean number of alleles, and observed and expected heterozygosity were estimated with Arlequin, version 3.1 (Excoffier et al. 2005). Further, we calculated the population estimates f for the inbreeding coefficient F_{IS} over nine loci according to Weir and Cockerham (1984) with a 95% confidence interval based on 1,000 bootstrap iterations using the Genetix software (Belkhir et al. 1996–2004). The presence of null alleles can bias the level of heterozygote frequency and thereby influence the inbreeding coefficient F_{IS}. As we suspected that our dataset contains some null alleles, we opted for a second estimate of the mating system that we calculated using the program RMES (David et al. 2007). RMES calculates selfing rates based on the multi-locus structure of the sample, i.e. the identity disequilibria among different loci and is not affected by null alleles.

To assess among-population genetic structure, we computed the global multilocus estimate of F_{ST} (θ) and the pairwise F_{ST} estimates for population pairs with FSTAT (Goudet 2001). We also used FSTAT to determine significance of the population differentiation using the log-likelihood statistic G. This statistic does not assume populations to be in HWE. Again, the presence of null alleles would bias the frequency of heterozygotes and render decreased genetic diversity, which would result in increased genetic differentiation among populations. We compared the global and pairwise θ estimates from FSTAT with the F_{ST} estimates obtained from the program FreeNA (Chapuis and Estoup 2007). FreeNA estimates null and visible allele frequencies and allocates a novel allele size to null alleles. Genotype frequencies are then adjusted and estimates of F_{ST} (Weir 1996) calculated on the basis of the visible alleles only, thereby ignoring the null allele state.

Based on the corrected allele frequencies obtained from FreeNA, we computed Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967) and performed 1,000 bootstrap iterations to assess the performance of the unrooted Neighbour-Joining tree using PHYLIP software (Felsenstein 2005).

To test for an effect of geographical distance on the population structure we calculated an isolation-by-distance test within the native region using the program IBD (Jensen et al. 2005). The parameter $F_{ST}/(1 - F_{ST})$ was used as measure of genetic distance (Rousset 1997). Geographic distance was calculated as the shortest distance between two populations.

As the 13 populations originated from 3 different geographical areas, we conducted a hierarchical AMOVA (Analysis of Molecular Variance; (Excoffier et al. 1992) to evaluate how the genetic variation was distributed among regions, among populations, and within populations. We used the Arlequin software on pairwise differences with 1,000 permutations (Excoffier et al. 2005).

Results

Genetic variation within populations

From a total of 468 possible linkage disequilibrium tests among-loci, 259 combinations could not be calculated because one of the test populations carried a fixed allele. Seven of the calculated locus-pair tests within populations showed significant linkage disequilibrium after applying a false discovery rate control (Benjamini and Hochberg 1995; Verhoeven et al. 2005). Out of the 117 possible population × locus combinations for Hardy–Weinberg testing, 55 tests could not be calculated because of a fixed or dominant allele. 20 of the remaining 62 tests showed significant deviations from Hardy–Weinberg equilibrium after controlling for false discovery rates (Benjamini and Hochberg 1995; Verhoeven et al. 2005). We carefully examined these loci in the respective populations and concluded that they most likely carry null alleles. Special care was taken to apply the appropriate analysis because null alleles can bias the expected heterozygosity and the statistics relying on this parameter. After correcting for null alleles, we included these cases in our analyses because they do not change the interpretation of the population genetic structure we see.

Number of polymorphic loci found per sample ranged from 1 to 9 loci (Table 2), native populations harboured more polymorphic loci (5–9 polymorphic loci, Table 2) than the introduced populations (1–2 loci, Table 2). The difference in polymorphism was also reflected in the mean number of alleles per population, which ranged from 1.67 to 4.22 alleles in the native and from 1.11 to 1.44 alleles in the introduced samples (Table 2). Seven out of the nine loci were fixed in all introduced populations, and only one population had two polymorphic loci. Figure 3 in the appendix shows the allele frequency distribution by region for the nine loci.

The Swiss populations showed significantly higher levels of genetic diversity than the Finnish samples (Mann–Whitney $U = 15.5$, $P < 0.05$). Expected heterozygosity values for Swiss samples were between 0.343 and 0.514, whereas Finnish population values ranged from 0.130 to 0.343 (Fig. 1). The introduced New Zealand populations showed very low genetic diversity with values from 0.006 to 0.052 (Table 2, Fig. 1). With only one exception among the New Zealand populations, the observed heterozygosity was always lower than the expected heterozygosity (Table 2). The exception was Lake Hayes (HY),

Table 2 Polymorphism data for 13 populations averaged over 9 loci

Region	Population	N	N_P	N_A	H_O	H_E	*s* (ML)	95% CI	*f* (*s*)	*f*	95% CI
CH	RS	30	8	3.22	0.401 (0.054)	0.489 (0.040)	**0.216**	[0.038–0.374]	**0.121**	**0.183**	[0.016–0.311]
CH	BH	40	9	4.00	0.473 (0.037)	0.511 (0.034)	0.000	[0–0.089]	0.000	0.075	[−0.021–0.132]
CH	EB	20	8	2.67	0.268 (0.053)	0.343 (0.051)	0.101	[0–0.439]	0.053	**0.226**	[0.005–0.343]
CH	SC	35	8	4.22	0.463 (0.036)	0.514 (0.038)	0.000	[0–0.130]	0.000	**0.101**	[0.005–0.165]
FIN	JA	20	7	2.44	0.206 (0.044)	0.321 (0.053)	0.128	[0–0.512]	0.068	**0.364**	[0.173–0.488]
FIN	VM	37	9	3.33	0.282 (0.036)	0.343 (0.032)	0.000	[0–0.281]	0.000	**0.179**	[0.032–0.288]
FIN	KO	20	6	1.67	0.141 (0.043)	0.157 (0.044)	0.000	[0–0.579]	0.000	0.100	[−0.201–0.299]
FIN	IS	18	5	1.67	0.125 (0.035)	0.130 (0.040)	0.099	[0–0.676]	0.052	0.038	[−0.201–0.239]
NZ	AL	40	1	1.22	0.006 (0.003)	0.006 (0.003)	NA	NA	NA	−0.007	[−0.044–0]
NZ	SE	39	1	1.22	0.010 (0.004)	0.021 (0.009)	NA	NA	NA	0.547	[−0.053–1.00]
NZ	HE	39	2	1.44	0.015 (0.005)	0.023 (0.008)	NA	NA	NA	0.355	[−0.075–0.633]
NZ	HY	32	1	1.11	0.018 (0.009)	0.017 (0.008)	NA	NA	NA	−0.067	[−0.167–0]
NZ	QT	35	1	1.22	0.035 (0.017)	0.052 (0.025)	NA	NA	NA	0.327	[−0.046–0.636]

N is the number of individuals, N_P the number of polymorphic loci and N_A, H_O, H_E are the mean number of alleles per locus, observed and expected heterozygosity, respectively. The numbers in the brackets give the standard error for these values. *s* (ML) stands for an estimate of selfing rate based on the ML method implemented in the program RMES with a confidence interval based on 1,000 bootstrap iterations. *f* (s) stands for the estimate of the inbreeding coefficient based on the selfing rate s. *f* stands for the estimate of the inbreeding coefficient F_{IS} (Weir and Cockerham 1984). Numbers in bold type are significantly different from zero

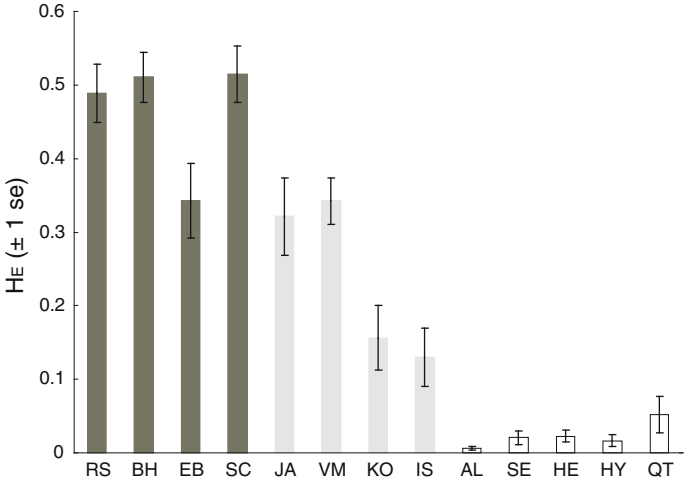

Fig. 1 Multi-locus estimates for expected heterozygosity H_E per population ±1 standard error. *Dark grey columns* represent the native Swiss and *light grey columns* the native Finnish populations. The *white columns* stand for the introduced New Zealand populations

where observed and expected heterozygosity was almost zero, and excess heterozygosity was not significantly different from zero (Table 2).

The traditional estimates of the inbreeding coefficient *f* were moderate to high, ranging from 0.038 to 0.364 in the native and −0.067 to 0.547 in the introduced populations (Table 2). The inbreeding coefficients can be translated into the selfing rate s by the formula $F_{IS} = s/(2 - s)$ (Crow and Kimura 1970) and would yield selfing rates of 0.074–0.534 in the native range. Using the identity disequilibria across loci, the program RMES calculated selfing rates in the range of 0–0.216. Whereas the traditional method resulted in significant homozygote excess for three Swiss and two Finnish populations, the robust multi-locus estimate of selfing only yielded one Swiss population to be out of Hardy–Weinberg equilibrium.

Estimates for the New Zealand populations were based on one to two polymorphic loci only, which unfortunately does not give enough resolution for mating-system analysis.

Genetic variation among populations

Populations were highly differentiated among and within regions. The global F_{ST} estimate for total sample differentiation was high, for both uncorrected (0.631; 95% CI: 0.587–0.670) and corrected data (0.618; 95% CI: 0.576–0.655). In the native range global F_{ST} estimate was less high, but reached a value of 0.341 (95% CI: 0.226–0.467). The single locus F_{ST} estimates showed that individual loci contributed similarly to this general result.

Population divergence was further assessed with pairwise θ values and revealed high variation within regions. Among the Swiss populations these ranged from 0.140 to 0.486, from 0.194 to 0.520 among the Finnish populations; and from −0.010 to 0.252 among the introduced populations (Table 3). Pairwise F_{ST} estimates between Swiss and Finnish populations showed a similar range of variation (0.342–0.716) as comparing the Swiss with the introduced populations (CH-NZ: 0.507–0.848), but the values of pairwise F_{ST} estimates were higher (Table 3). The comparison between the Finnish and the introduced

Table 3 Pairwise θ values on the lower diagonal based on 9 loci

	RS	BH	EB	SC	JA	VM	KO	IS	AL	SE	HE	HY	QT
RS		*	*	*	*	*	*	*	*	*	*	*	*
BH	0.292		*	*	*	*	*	*	*	*	*	*	*
EB	0.486	0.417		*	*	*	*	*	*	*	*	*	*
SC	0.259	0.140	0.345		*	*	*	*	*	*	*	*	*
JA	0.424	0.342	0.502	0.386		*	*	*	*	*	*	*	*
VM	0.436	0.391	0.522	0.434	0.227		*	*	*	*	*	*	*
KO	0.548	0.497	0.686	0.498	0.438	0.194		*	*	*	*	*	*
IS	0.580	0.520	0.716	0.538	0.520	0.394	0.474		*	*	*	*	*
AL	0.739	0.611	0.848	0.568	0.836	0.791	0.933	0.942		ns	ns	ns	*
SE	0.718	0.585	0.824	0.541	0.813	0.772	0.918	0.924	0.027		ns	ns	*
HE	0.722	0.593	0.826	0.552	0.816	0.776	0.918	0.925	0.012	-0.004		ns	*
HY	0.698	0.562	0.804	0.522	0.793	0.754	0.913	0.916	0.037	0.012	-0.010		*
QT	0.692	0.556	0.795	0.507	0.779	0.748	0.891	0.898	0.252	0.167	0.180	0.177	

Population differentiation at the 5% nominal level on the upper diagonal (excluding locus A2)

populations showed less variation (FIN-NZ: 0.748–0.942; Table 3) and extremely high levels of differentiation. We compared the traditional F_{ST} estimates with the ones corrected for null alleles and found them to vary only little (difference: −0.033 to 0.041; mean: 0.008; standard deviation: 0.018) and therefore continued with the traditionally obtained estimates. We examined pairwise population differentiation with log-likelihood tests (excluding locus A2, which did not amplify for population SC) and found all but six population pairs to be significantly different at the 5% level. The six population comparisons originate from the introduced range. A Neighbour-Joining tree based on Cavalli-Sforza chord distances confirmed the three regions to be significantly divergent (Fig. 2). The split between the Swiss and the Finnish populations was supported with 87% and the split between the Swiss and the New Zealand population with 100% out of 1,000 bootstrap iterations.

The Mantel's test for isolation by distance was significant ($r = 0.508$, $P = 0.012$), if both native regions were included in the dataset, but we found no evidence when examining the relationship within each region (Mantel's test, CH: $r = -0.077$, $P = 0.55$; FIN: $r = -0.898$, $P = 0.99$).

Hierarchical analysis showed that 55% of the observed variation was distributed among the three geographic regions. 13.56% of the genetic structure divided the populations within regions, whereas the differentiation among individuals within samples was 31.43%. The three fixation indices F_{ST} (0.686, proportion of total variance shared within populations), F_{SC} (0.301; proportion of total variance that remains among populations within regions) and F_{CT} (0.550; proportion of variance that is found among the three regions) were all highly significant after 1,023 permutations.

Discussion

Colonization events are often followed by a loss of genetic diversity. However, genetic diversity is a key proxy for a species' ability to adapt to novel environmental variation. Our study shows that small population size and limited gene flow during the colonization process probably play a vital role for the population genetic structure in this hermaphroditic freshwater snail. Our results demonstrate that the populations of Switzerland show characteristics of ancestral populations (high genetic diversity, high genetic structure),

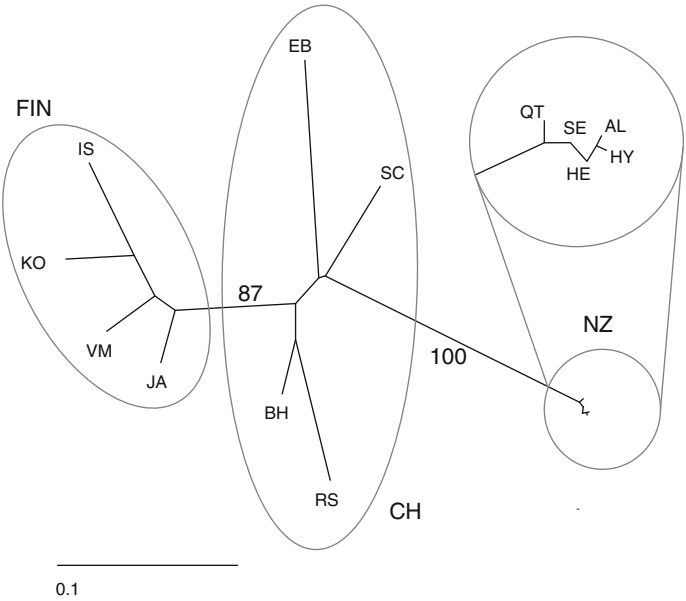

Fig. 2 Unrooted Neighbour-Joining tree of 13 populations originating from Switzerland (CH), Finland (FIN) and New Zealand (NZ) based on Cavalli-Sforza genetic distances. *Numbers* give percentage of support assessed through 1,000 bootstrap iterations

while the populations of Finland show characteristics of a historical range expansion (lower genetic diversity, high genetic structure) and the populations of New Zealand show characteristics of a single or a few recent invasion events (very low genetic diversity and very little genetic structure).

Overall, we found the level of genetic diversity to correlate with the age of the populations. In the native, non-glaciated Swiss populations the genetic diversity was significantly higher than in the Finnish populations. Whereas we expect to find the highest genetic diversity in the most ancestral populations, the observed differentiation among the Swiss populations seems substantial, especially if we consider that most of the pairwise F_{ST} values between Finnish and Swiss populations are about equally high as the differentiation among the Swiss populations.

One possible process generating such a strong local genetic structure in the native range as observed in Switzerland might be found in the basic ecology of *Lymnaea stagnalis*. In Switzerland the species is especially common in small temporary/semi-permanent ponds (Jokela, unpublished data). It is not unusual that such populations exhibit extinction-colonization dynamics where empty ponds are being recolonized by migrants from the regional population pool. Although we did not find support for significant on-going self-fertilization in our population samples, self-fertilization in these hermaphroditic snails is possible and occurs in isolation (Puurtinen et al. 2007). Both, self-fertilization, and the option of sperm storage allow establishment through a single individual after an extinction event and would enhance the genetic stochasticity associated with colonization. In such a system, initial colonization by only few lineages of the regionally diverse gene pool may lead to a strong local population genetic structure.

In Finland the regional gene-pool might not be as diverse, because the whole system is much younger. We see lower levels of genetic diversity than in the Swiss populations but

an equal level of differentiation, expressed in either pairwise F_{ST} values or Cavalli-Sforza chord distances (Table 3, Fig. 2). In Fennoscandia the last glacial maxima was reached 21,000–17,000 years BP (Andersen and Borns 1997; Svendsen et al. 2004) and after the ice-sheet had retreated the landmass of Finland was still rising. Therefore, suitable habitats for *L. stagnalis* in Finland are unlikely to be older than 10,000 years, and may have been colonized for a much shorter period of time. The diminished genetic diversity conforms to the expectations for populations at the edge of a species' distribution. Post-glacial range expansion should be accompanied by re-occurring colonization events and subsequent genetic founder effects (Hewitt 1996, 1999; Avise 2000). Studies for some species show that populations in areas at the edge of the range expansion harbour lower levels of genetic diversity than in the core areas (Bernatchez and Wilson 1998). Additionally, we find a pattern of rather strong divergence despite the more permanent type of habitat the snails inhabit in Finland. Considering that the populations in this study come from separated waterbodies we presume that the differentiation within Finland could be a historical signature of divergence after colonization.

The isolation by distance pattern across the native range is strongly driven by the comparisons between the two rather distant native regions. In Switzerland geographic distances between populations range from 15 to 47 km, in Finland populations are 30–325 km apart and between country pairs range from 1,971 to 2,299 km distance. We argue that our dataset does not provide evidence for isolation by distance because of the missing sample pairs with intermediate distances. Moreover, if we look at the within country comparisons, the association between geographic and genetic distance does not hold true. Although a lacking isolation by distance pattern on a local scale does not impede this association to come through on a larger scale, the non-existent or negative association of those parameters in the native regions lead to the conclusion that migration between local populations is low and drift is probably a strong force shaping the local population structure.

In the non-native range a population bottleneck is a likely start for a new population. More than 100 years post-invasion and an equal number of generations the introduced populations in New Zealand show very little genetic diversity with a maximum average of 1.44 alleles per microsatellite locus. Even in the initial data set with 14 loci this parameter did not exceed 1.50. The low genetic diversity and the relatively low degree of divergence among these populations suggests that the invasion stems from a severely inbred ancestral stock or possibly went through several sequential bottlenecks. One can also entertain the idea that the Acclimatization Societies unconsciously selected for the fittest individuals that survived the shipping from Europe to New Zealand. We hypothesize that the low observed genetic variation is due to sequential bottlenecks and strong effects of genetic drift. If post-invasion mutations created the observed mutant alleles, we would expect these to be the same size of the dominant allele plus or minus one repeat motif which is not the case for both polymorphic loci within New Zealand. For this particular invasion we know that it is a recent event, and not surprisingly the populations cluster tightly together in the distance tree when compared to the native range. If the populations continue to thrive in New Zealand one can expect new genetic variation to emerge, but it would take a very long time for a similar level of genetic differences to develop among New Zealand populations than those observed among populations in the native range. Such divergence would probably require several new invasion events and following admixture, which is a very unlikely scenario taken the present regulatory measures in place in New Zealand. Rapid loss of genetic diversity has been observed in some aquatic invasions (Hauser et al. 1992; Städler et al. 2005; Roman and Darling 2007), but the opposite, an increase in genetic

diversity seems equally likely in the aquatic habitat (Woodruff et al. 1985; Roman and Darling 2007; Facon et al. 2008; Gillis et al. 2009).

In many snail species, mating-system clearly is a decisive factor for successful invasion. Many of the current invasive snail species are prosobranchs (*Potamopyrgus antipodarum*, *Melanoides tuberculata*, *Tarebia granifera*) due to their ability of asexual reproduction. Pulmonate species on the other hand are hermaphrodites, which generally show a tendency towards outcrossing or selfing in their native populations. *L. stagnalis* has been classified as outcrossing (Cain 1956; Coutellec-Vreto et al. 1994; Puurtinen et al. 2007) and seems to be an outlier when looking at its invasion success. Most of the studied invasive pulmonate snails have been shown to be preferentially selfing in their native range (*Lymnaea truncatula*, *Biomphalaria pfeifferi*). All our study populations in New Zealand and in the native range, except one, were in Hardy–Weinberg equilibrium. We hypothesized above that a single individual may secure the establishment of a population, and can together with founder events also inflict strong population genetic structure. Observed inbreeding coefficients in Finnish populations were in the same range as the estimates that Puurtinen et al. (2004) reported for eight Finnish populations, but differed markedly when the estimates were corrected for the presence of null alleles. Considering that the microsatellite data of molluscs are often encumbered by null alleles, our data suggests that inbreeding does not influence the resulting population structure. This suggestion is consistent with those earlier studies that suggest that the hermaphroditic *L. stagnalis* is able to self-fertilize, but has a strong preference for outcrossing (Cain 1956; Puurtinen et al. 2007).

In conclusion, the effects of colonization, lack of significant gene flow between populations and environmental stochasticity seem to foster significant population genetic divergence in the native range of this freshwater snail. Populations in northern Europe, where post-glacial range expansion has taken place, are similar in divergence as central European populations, but show lower genetic diversity. The genetic signature of the invasive populations differs from the native range by highly reduced genetic diversity and low degree of differentiation. We conclude that the population genetic structure of invasive *L. stagnalis* is fundamentally different to the native range and is likely to remain so, taken that new invasion events do not take place.

To better explain the strong structuring in the native populations we propose that future studies should test the hypothesis that frequent extinction-colonization events and stochastic recolonization of habitat patches together with low continuous gene flow between populations are the most important factors for explaining the observed strong genetic structure. Additionally, a comparison with more slowly evolving phylogenetic markers might allow a more precise evaluation of divergence between wide geographic regions like Finland and Switzerland. However, the invasive populations of New Zealand show exactly the predicted population genetic pattern that is expected from an invasion event with small propagule pressure.

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Appendix

See Table 4 and Fig. 3.

Table 4 Characterization of six microsatellite loci for *Lymnaea stagnalis* based on four Swiss populations (20–40 individuals)

Locus	Repeat motif	Accession no.	Primer sequence (5′–3′)	Size (bp)	N_A	H_E	H_O
2k27	$(TG)_{10}X(TG)_2$	EF208747	F: CGACACATTATTTCGAAGGTG R: ATCCATTCCACTGTGGCCTA	114–122	2–4 (4)	0.05–0.577	0.05–0.441
2k11	$(CA)_9$	EF208748	F: TGTCTCCCATTTCCAACTCA R: CGACCCCCTTGTTCCTTTAC	141–154	3–5 (6)	0.262–0.745	0.037–0.6
2k33	$(TG)_2X(TG)_9$	EF208749	F: ACATCGCTTTGGTGTGTTCA R: CCTCTGTGCAAGACCTGTCA	108–118	2–5 (6)	0.05–0.624	0.05–0.546
2k68	$(CA)_{21}$	EF208750	F: TTGTCAGTGCGGGGTGTGTATC R: CAGTAGGAGGAACATCTCAAAGACG	159–191	2–10 (12)	0.508–0.847	0.4–0.875
2k46	$(GA)_{21}X(GA)_{24}$	EF208751	F: CGAATGTCTTGATGGCATGT R: GTTGTCACCAGCAACCATTC	110–147	2–5 (10)	0.135–0.72	0–0.214
2k42	$(CA)_7X(CA)_3X(CA)_5$	EF208752	F: TGCAACCTTAACCATGCAAG R: GATTCAAGATGGGCAACCTC	147–161	3–4 (5)	0.126–0.632	0.132–0.667

The table shows the locus name, repetitive sequence, accession number in Genbank, primer sequences, range of product sizes, N_A as observed numbers of alleles (population range and total number) and the range of heterozygosity values (for H_O and H_E). Locus 2k46 was excluded from the study due to null alleles

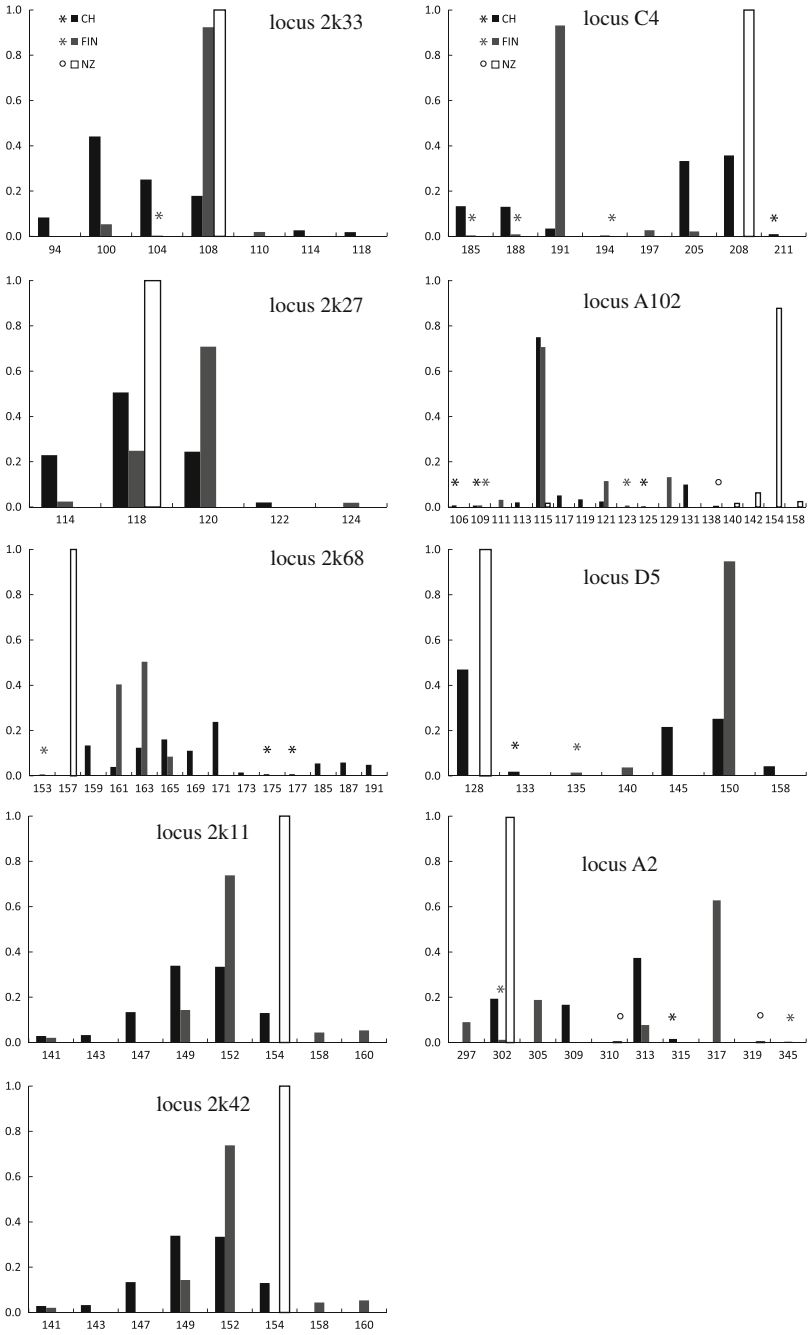

Fig. 3 Allele frequencies of nine loci by region. *Black bars* and *stars* depict Switzerland (CH), *grey bars* and *stars* stand for Finland (FIN) and *white bars* or *circles* stand for New Zealand (NZ). Allele size in bp is indicated on the x-axis Allele size (bp) and frequency on the y-axis

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